

## The karyotype and taxonomic status of *Cryptomys amatus* (Wroughton, 1907) from Zambia (Rodentia, Bathyergidae)

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> Receipt of Ms. 05. 08. 1997 Acceptance of Ms. 02. 12. 1997

Key words: Cryptomys, Common mole-rats, Bathyergidae, karyotype, taxonomy

Unlike the whole family Bathyergidae, with rather well-understood intrafamiliar systematic relationships but unclear sister-group affinities to other lineages within the Hystricognathi (Honeycutt et al. 1991), the genus *Cryptomys* per se poses no serious problem for taxonomists, yet its intrageneric systematics are difficult and far from being resolved. Thus, for example, the number of species described within the genus was highly author-dependent, ranging from 3 (Nowak and Paradiso 1983) to as many as 44 (Allen 1939) or even 49 (Ellerman 1940). Recently, Honeycutt et al. (1991) have suggested 7 valid species, and their classification has been currently widely accepted (e.g., Woods 1993). Nevertheless, results of karyological and biochemical studies on two yet unnamed *Cryptomys* species from Zambia (Burda et al. 1992; Filippucci et al. 1994, 1997), suggest a need for a revision of the established taxonomic scheme.

Generally, information on karyotypes within the genus *Cryptomys* is scant, the only known data being those on *C. foxi* (Thomas, 1911) (WILLIAMS et al. 1983), *C. hottentotus hottentotus* (Roberts, 1913), *C. h. natalensis* (Roberts, 1913), and *C. damarensis* (Ogilby, 1838) (Nevo et al. 1986), *C. darlingi* (Thomas, 1895) (AGUILAR 1993), *C. mechowi* (Peters, 1881) (Macholán et al. 1993), and two unnamed species from Itezhi-Tezhi and Lusaka, respectively (Burda et al. 1992). Differentially stained chromosomes were studied to even less extent (Nevo et al. 1986; AGUILAR 1993; Macholán et al. 1993).

According to Honeycutt et al. (1991), Cryptomys amatus, originally described as Georychus amatus by Wroughton (1907), and later ascribed to the genus Cryptomys by Allen (1939), is a subspecies of C. hottentotus Lesson, 1926. In this study, we present for the first time results of a karyotypic study on small common mole-rats collected at the type locality of C. [hottentotus] amatus (Wroughton 1907; Moreau et al. 1945). The diploid chromosomal number and morphology of the chromosomes led us to ascribe these specimens to the distinct species, C. amatus, karyotypically separated from other Cryptomys species.

In total, 14 individuals (9 males, 5 females) were collected by the road to Chibale, Zambia (S 13°35′; E 30°05′), 1300–1500 m a.s.l. All but a single animal were adult, the only exception being a subadult male. Two males (one "grey" and one "brown", see below) and one female ("grey") were karyotyped. Mitotic metaphases were obtained directly from bone marrow. Slides were differentially stained using the trypsin digestion (G-banding) technique by Seabright (1971), and the C-banding technique by Sumner (1972). Nucleolus organizer regions (NORs) were visualized by the silver-staining method of Howell and Black (1980).

All the animals were captured in cultivated fields, savannah-woodland, and/or savannah-bushland. The collecting site represents a mesic habitat with mid-July temperatures ranging from 10.0 to 12.5 °C and a mean annual rainfall of 944 mm (according to records of the Serenje Climatic Station). The giant mole-rat (*C. mechowi*) occurs sympatrically in this area.

Two colour variants were found at the same site: 8 individuals (5 males, 3 females) were dark grey, whereas 6 individuals (4 males, 2 females) were tan or brown. As no sexual dimorphism in body weight was found within both colour groups, sexes were pooled in the subsequent analyses. The brown animals were heavier ( $w = 73.2 \, g$ ; SD = 10.01; range  $52-71 \, g$ ) than the grey ones ( $63.3 \, g$ ; SD = 8.12;  $61-88 \, g$ ; the subadult male excluded), but the difference was insignificant (ANOVA: F = 3.871, p = 0.075). Most animals, irrespective of their overall coloration, had a small or even missing white forehead patch and only few individuals revealed a large patch; in some animals, a white spot was also found on the mentum or belly. Several animals had a rusty-red or brown mentum similar to *C. mechowi*. The infraorbital foramen was elliptical to triangular.

The diploid chromosomal number of all the specimens examined (both "grey" and "brown") was 2 n = 50. Twenty-two pairs of autosomes were biarmed, mostly meta- or submetacentric, except for the first pair which was subtelocentric. Pairs Nos. 17 and 24 were acrocentric but in one of the males, the former appeared to be heteromorphic, one of the elements being subtelocentric rather than acrocentric. The X chromosome was large metacentric, whereas the Y was small and acrocentric (NF = 96; NFa = 92).

The G-banding and C-banding patterns are shown in figure 1. The Y chromosome revealed a rather inconspicuous G-banding pattern while being wholly positively stained in C-banded metaphases. The X possessed only a tiny centromeric block of heterochromatin. Conversely, most autosomes displayed, in addition to the centromeric heterochromatin, apparent telomeric C-bands (Fig. 1).

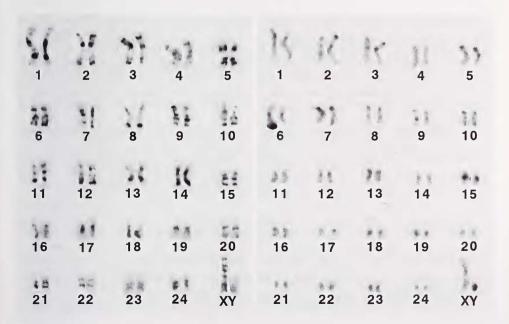


Fig. 1. G-banding (left) and C-banding (right) pattern of the karyotype of a male MM 900 ("brown" variant).



**Fig. 2.** AgNOR-stained karyotype of a female MM 903 ("grey" variant). Large arrowheads show apparent NORs which were proven to be active virtually in all examined metaphase spreads; in the pair of small autosomes (small arrowheads), the organizers were usually weakly stained or inactive.

Three pairs of autosomes appeared to possess NORs. However, in the smallest NOR-bearing pair (No. 24), the organizers were usually very pale and frequently not active at all. The NORs were located telomerically on the smallest (acrocentric) autosomes and on the short arms of pair No. 1 as well as on a medium-sized submetacentric pair (Fig. 2).

In comparison with two other Zambian species of common mole-rats (Burda et al. 1992), the specimens examined in the present study were generally smaller, having thicker and more velvety pelages, exhibiting age-independent colour polymorphism, and a great variation in size and shape of the white head spot. However, both the inter- and intraspecific differences were quantitative rather than categorical. Given considerable polymorphisms in the traits traditionally considered diagnostic within the genus *Cryptomys*, it is hard to provide any sound diagnostic keys based solely on the morphological characteristics studied.

The karyotype of C amatus represents one of the lowest known number of chromosomes within the Bathyergidae, the diploid number of C mechowi with 2n = 40 (Macholán et al. 1993) being the only exception. Other species of the genus hitherto studied karyologically have shown higher diploid numbers: the "Itezhi-Tezhi" and the "Lusaka" species from Zambia with 2n = 58 and 2n = 68, respectively (Burda et al. 1992), C foxi from Cameroon 2n = 66 and 2n = 70 (Williams et al. 1983), and C damarensis from Namibia and Botswana 2n = 74 and 2n = 78, respectively (Nevo et al. 1986). Among the taxa with chromosome numbers closest to that of C amatus are C darlingi from Zimbabwe (Aguilar 1993), and C h. hottentotus and C h. natalensis from South Africa (Nevo et al. 1986), with 2n = 54. Irrespective of the same diploid number, however, the morphologies of chromosomes in C darlingi and C hottentotus are very different.

The distinct diploid number and morphology of the chromosomes (see Macholán et al. 1993 for a review) suggest *C. amatus* can be considered a separate species, karyotypically fairly well differentiated from other species of the genus. Basic characteristics of all the known karyotypes among common mole-rats indicate that these species are geneti-

cally diversified and evolutionarily clearly separated. This seems to be corroborated also by results of allozyme and molecular studies (Honeycutt et al. 1987; Nevo et al. 1987; FILIPPUCCI et al. 1994, 1997). However, it is not possible to draw any conclusion as to the closest relatives of *C. amatus* and the evolutionary interrelationships within the genus as a whole solely on the chromosomal data owing to the scarcity of available high-resolution G-banded karyotypes.

## Acknowledgements

This work was partly supported by a travel grant of the DAAD for A. Scharff. We thank Dr. D. Kock for his critical comments on an earlier version of the manuscript.

## References

- AGUILAR, G. H. (1993): The karyotype and taxonomic status of *Cryptomys hottentotus darlingi* (Rodentia: Bathyergidae). S. Afr. J. Zool. **28**, 201–204.
- ALLEN, G. M. (1939): A Checklist of African Mammals. Harvard Coll., Cambridge, Mass.: Bull. Mus. Comp. Zool.
- Burda, H.; Filippucci, M. G.; Macholán, M.; Nevo, E.; Zima, J. (1992): Biological, allozyme, and karyotype differentiation of African mole-rats (*Cryptomys*, Bathyergidae) from Zambia. Z. Säugetierkunde, Suppl. 57, 11–12.
- ELLERMAN, J. R. (1940): The Families and Genera of Living Rodents. London: Trustees of the Brit. Mus. Nat. Hist.
- FILIPPUCCI, M. G.; BURDA, H.; NEVO, E.; KOCKA, J. (1994): Allozyme divergence and systematics of common mole-rats (*Cryptomys*, Bathyergidae, Rodentia) from Zambia. Z. Säugetierkunde **59**, 42–51.
- FILIPPUCCI, M. G.; KAWALIKA, M.; MACHOLÁN, M.; SCHARFF, A.; BURDA, H. (1997): Allozyme differentiation and systematic relationship of Zambian Giant mole-rats, *Cryptomys mechowi* (Bathyergidae, Rodentia). Z. Säugetierkunde **62**, 172–178.
- HONEYCUTT, R. L.; ALLARD, M. W.; EDWARDS, S. V.; SCHLITTER, D. A. (1991): Systematics and evolution of the family Bathyergidae. In: The Biology of the Naked Mole-Rat. Ed by P. W. SHERMAN, J. U. M. JARVIS, and R. D. ALEXANDER. Princeton: Princeton Univ. Press. Pp. 66–96.
- HONEYCUTT, R. L.; EDWARDS, S. V.; NELSON, K.; NEVO, E. (1987): Mitochondrial DNA variation and the phylogeny of African mole rats (Rodentia: Bathyergidae). Syst. Zool. 36, 280–292.
- Howell, W. M.; Black, D. A. (1980): Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a one-step method. Experientia 36, 1014–1015.
- Macholán, M.; Burda, H.; Zima, J.; Míser, I.; Kawalika, M. (1993): Karyotype of the giant mole-rat, *Cryptomys mechowi* (Rodentia, Bathyergidae). Cytogenet. Cell Genet. **64**, 261–263.
- MOREAU, R. E.; HOPKINS, G. H. E.; HAYMAN, R. W. (1945): The type-localities of some African mammals. Proc. zool. Soc. Lond. 115, 387–429.
- Nevo, E.; Ben-Shlomo, R.; Beiles, A.; Jarvis, J. U. M.; Hickman, G. C. (1987): Allozyme differentiation and systematics of the endemic subterranean mole rats of South Africa. Biochem. Syst. Ecol. 15, 489–502.
- Nevo, E.; CAPANNA, E.; CORTI, M.; JARVIS, J. U. M.; HICKMAN, G. C. Karyotype differentiation in the endemic subterranean Mole rats of South Africa (Rodentia, Bathyergidae). Z. Säugetierkunde 51, 36–49.
- Nowak, R. M.; Paradiso, J. K. (1983): Walker's Mammals of the World. 4<sup>th</sup> ed. Baltimore, London: John Hopkins Univ. Press.
- SEABRIGHT, M. (1971): A rapid banding technique for human chromosomes. Lancet 2, 971–972.
- SUMNER, A. T. (1972): A simple technique for demonstrating centromeric heterochromatin. Expl. Cell. Res. **75**, 304–306.
- WILLIAMS, S. L.; SCHLITTER, D. A.; ROBBINS, L. W. (1983): Morphological variation in a natural population of *Cryptomys* (Rodentia: Bathyergidae) from Cameroon. Ann. Mus. Roy. Afr. Centr., Sc. Zool. **237**, 159–172.

- Woods, C. A. (1993): Suborder Hystricognathi. In: Mammal Species of the World. A Taxonomic and Geographic Reference. Sec. ed. Ed by D. E. Wilson and D. A. Reeder. Washington, London: Smithsonian Inst. Press. Pp. 771–806.
- WROUGHTON, R. C. (1907): On a collection of mammals made by Mr. S. A. Neave in Rhodesia, North of the Zambezi, with field notes by the collector. Manchester memoirs 51, 1–39.
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